

## GENETIC STUDIES IN SPACE

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G. P. Parfenov

**ABSTRACT.** Review of Soviet and foreign papers on genetic studies in space covering the period from the late twenties through 1965. Discussed specifically are the results of free-balloon, rocket, and satellite experiments with microorganisms, plants, and animals. A brief analysis of these results is given.

Genetic experiments in space have already been going on for more than /140\* three decades, if we count studies conducted in the upper atmosphere, which is equivalent to space in many respects. Naturally a great amount of material has been accumulated during this time. The purpose of this article is to give the most complete and generalized possible review of these investigations.

Since genetic research is the subject, the review should include only studies based on hybridological analysis of offspring or cytological study of the material carriers of inheritance. In many cases, however, it is difficult to distinguish genetic studies from physiological, biochemical, and radiobiological studies, such as when analyzing the survival of microorganisms or the induction of sterility. Such investigations also will be considered.

The experiments were conducted by exposing biological objects carried aloft in balloons, rockets, and artificial earth satellites. The type of exposure determines some distinguishing characteristics of the experiments (altitude and duration of flight, the combination of factors affecting the object, etc.). Therefore, the experiments will be arranged for review according to the method used for exposure of the material, although this will somewhat mar the chronology of exposition.

Interest in genetic investigations in space was the result of two discoveries: the discovery of ionizing cosmic rays at the earth's surface, and the proof beyond doubt in the late 1920's of the mutagenic effect of ionizing radiation. It was theorized that spontaneous mutations in organisms are not actually spontaneous, but result from the effect of cosmic radiation. However, attempts to verify this hypothesis experimentally usually gave negative results. The method used was to place the experimental series of objects either underground [1-5] or in the mountains [6-11]. In two studies [9-11] an effect was noted, but the authors themselves question both the reality of the effect and its origin.

Studies in which biological objects (bacteria, lower fungi, *Drosophila*, mice) were placed under shields of various thicknesses or were left unprotected/141 gave more definite results. When screen thickness was such as to form a shower of cosmic particles by the Rossi effect, genetic changes were usually observable [12-17].

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\*Numbers in the margin indicate pagination in the foreign text.

# 1. Experiments in Balloons

The poor results of studies conducted on the ground brought many leading geneticists to the opinion that the question of the genetic effect and evolutionary significance of cosmic radiation would have to be solved by lofting suitable biological objects on stratospheric balloons. In particular, such ideas were expressed by N. Kol'tsov, H. Müller and G. Nadson at the All-Union Conference on Study of the Stratosphere in 1935 [18-20].

A suitable experiment was devised as early as 1935. The stratospheric balloon "SSSR-1-bis", which attained an altitude of 15,900 m, carried male *Drosophila* of the normal Nal'chik line, which were analyzed after return to earth for the presence of recessive lethals in the sex chromosome. No statistically reliable difference was found in the incidence of lethals in the experimental and control groups. On the basis of this experiment, Friesen concluded that cosmic radiation plays an unimportant role in evolutionary processes on earth [21-23].

A similar experiment was carried out in the United States in 1935 on the balloon "Explorer 2". Besides male *Drosophila*, which were analyzed for recessive lethals in the sex chromosome and one of the autosomes, the "Explorer 2" carried fungus spores. No differences were found between experimental and control groups of either *Drosophila* [24] or fungus spores [25].

Thereafter studies of the upper atmosphere by balloons carrying biological objects were suspended for a long time. Some geneticists called these experiments the "swan song" of genetic studies in space. However, early in the 1950's biological balloon studies were revived with increased intensity. This is obviously due to the fact that the ceiling for balloons had risen to 20-30 km, that is, they began to reach the upper limit of penetration by heavy primary cosmic particles. In addition, the duration of balloon flights increased to approximately one day and the exposure of material accordingly increased in duration.

A considerable number of balloons were released in the United States from 1951 to 1958 for the Skyhook, Man High, and Stratolab projects. One of the main objectives of these balloon flights was to study the biological effect of the heavy component of primary cosmic radiation. The cytological research method was used extensively in these experiments. The experimenters counted the number of tracks left by heavy particles in sections of tissues of living mammals carried aloft. Analyses were made mainly of nerve, skin, and retinal tissue. Mice, hamsters, rats, rabbits, guinea pigs, and cats were used in the experiments [26-33]. Altogether a great many experiments were made, although many flights ended in failure; however, in no instance was it possible to evaluate the effect of cosmic radiation or even detect its effect on tissue. The authors concluded that this method is inadequate for the study of the biological effect of cosmic radiation [26,31,33].

Mammalian and human tissue (usually skin) *in vitro* was also frequently exposed on balloons. Topical recording of cosmic radiation effects was usually <sup>/142</sup> done at the same time, using grafts impregnated with photoemulsions by Eugster's [34] and Schaefer's [35] method. In some cases the skin grafts were regrafted

onto the donors [28,34,36-41]. The method of photoemulsion-impregnated skin grafts provided an adequate count of the number of cosmic ray particles passing through the grafts. However, it did not clarify the specific biological effect of the heavy component of cosmic radiation. In some experiments the effect noted was difficult to explain. For example, Eugster, who placed his experimental material on balloons and his controls in a tunnel or at the summit of the Jungfrau, found flight to have a stimulating effect on cellular activity of the grafts [41].

In the opinion of investigators conducting balloon experiments, Chase's [42] method of recording the depigmentized hairs of black mice (C57b1 line) was the most successful. This method always yielded a noticeable difference between the experimental and control material [28,29,31-33,42-44]. On the basis of the nature of the effect and the cell mechanism of pigment formation, Chase felt that a single heavy particle can adversely affect 50 cells or more. It should be noted, however, that all black mice have some depigmentized hairs, which increase in number with age. Chase himself [44] feels that his data required further checking and confirmation.

Emulsion simulators of living tissues (phantoms), developed by Yagoda, were of definite importance for estimating cosmic radiation doses absorbed during balloon flights. It was noted that the frequency of heavy particle tracks increases exponentially with a decrease of atmospheric depth [30,45,46].

On many flights eggs of the dwarf shrimp *Artemia salina* were used. Survival rate of the eggs was determined. In many cases the experiments were backed up by photoemulsion control [30-34,36,37,40]. Death of the eggs due to direct bombardment by heavy cosmic ray particles was noted several times. One of Eugster's experiments [36] yielded the following results: after flight [28-30 km] the egg death rate reached 100%; compared with 91% for the underground controls (Simplon tunnel) and 4% for the controls at the earth's surface. In view of these data, it is difficult to agree that a significant part of the effect was due to cosmic radiation. No flight effect could be discovered in the germination and growth dynamics of radish seeds [28,29,40]. An experiment in which barley seeds (280 seeds) were exposed for 48 hr at an altitude of 30-32 km (on two successive flights) resulted in increased sterility, a smaller number of ears and fewer grains to the ear, and the appearance of chlorophyll mutants. The data on the latter are not statistically reliable. A total of 8539 plants were analyzed in the first to fifth generations. Twenty-five heavy particle hits into the center of the grain and 18 marginal hits were counted. It is interesting that the increase in sterility of the grain after heavy particle penetration was less significant than that noted after penetration by  $\gamma$ -rays [47-49].

Several balloon studies were made with neurospora (conidia and paper saturated with spores) and orthopteran sperm and egg cells [50-52]. The experiments with neurospora usually showed a positive effect [30,33,51]. It should be noted that no detailed description of experiments on these objects exists in the available literature. In all probability they were not completed.

Poliomyelitis virus, T<sub>4</sub> phage, and *Penicillium roqueforti* spores exposed /143

on a balloon (34 km, 6 hr) with minimum cosmic ray shielding (38  $\mu$ m Al) showed no changes in survival, virulence, or bacteriostatic activity [53-54].

Sullivan and Thompson exposed unprotected insect eggs at an altitude of about 15 km. The eggs of *Prodenia ornithogalli* died, presumably from the cold. At the same time, the eggs of *Malacosoma americanum* survived [55]. No biological changes were observed after landing in domestic flies and fleas which had been in the balloon gondola [56].

In 1959, Pipkin and Sullivan [57], testing Müller's idea that the heavy cosmic ray component must primarily induce chromosome and not gene mutations [58], used 10,761 *Drosophila* larvae in a suitable balloon experiment. The flight lasted 16 hr at an altitude of 24 km. The method permitted an alternate count of gene and chromosome mutations arising in a single genetic system. There were no differences in the ratio of the two types of mutations. However, the investigators felt that developing lethal chromosome mutations may have escaped observation with this method.

In another experiment [59] male *Drosophila* were flown for 12 hr at an altitude of 30 km; the dose of cosmic radiation was 0.25 mrad. The frequency of recessive lethals in the experimental flies increased, but this increase was statistically unreliable and no translocations were observed; at the time, the frequency of induced dominant lethals was about 10%. The effect disappeared after 9 days, that is, with the onset of spermatogonia separation. It should be noted that after flight the males were bred individually and the copulations recorded. Nevertheless, it is difficult to agree with the authors that development of the dominant lethals was caused by cosmic radiation.

Experimental and control specimens revealed no differences in either cell survival or chromosome aberrations when mono-layer cell cultures of mammalian and human tissues were exposed on balloons [60-61].

The only balloon experiment on living mammals using genetic research methods gave negative results [62]. Mice (35 males and 54 females) were flown for 24 hr at an altitude of 24-33 km. No differences were observed between the experimental and control animals with respect to fertility, fecundity, cytology of the gonads, the gametogenic cycle, or longevity.

In the genetic experiments conducted on balloons, the emphasis was on study of the effectiveness of cosmic radiation. The following general statement may be made concerning these investigations. As a rule the genetic effect (if any) was insignificant in absolute terms, whether or not the difference between experimental and control specimens was statistically reliable. Thus, upper atmosphere flights present no genetic hazard provided very large populations are not involved.

Biological dosimetry by genetic methods of cosmic radiation under these conditions is exceptionally complex because the doses encountered approximately coincide with the detection threshold of the most modern research methods. In addition, conducting such experiments involves the problem, not easily solved, of distinguishing changes caused by specific flight factors from changes caused

by nonspecific factors. The latter are introduced by unavoidable differences in maintenance conditions for experimental and control specimens, or by the temporary exposure of the experimental series to uncontrolled conditions. In the /144 experimental material this causes, firstly, increased phenotype variability, which is difficult and at times impossible to distinguish from genotype variability, and secondly, an increase in true genetic variability (for example, due to temperature).

## 2. Experiments on High-Altitude Rockets

Considerably fewer genetic experiments were carried out on rocket flights than on balloons. Moreover, the emphasis of these investigations shifted somewhat. Besides cosmic radiation, much attention was given to clarification of the mutagenic activity of other factors accompanying rocket flight: vibration, accelerations, and weightlessness.

In the United States from 1946 to 1952, "V-2" and "Aerobee" rockets were launched carrying lower fungi, *Drosophila*, mice, and monkeys. However, most of these launchings were unsuccessful and the biological objects died [63-66]. Flights of rockets carrying biological objects were continued from 1953 to 1957 [64,67], but few genetic studies were made.

An experiment using the flour beetle *Triboleum castaneum* gave negative results [68]. The rocket flight lasted 30 min and reached an altitude of 85 km. The survival rate of the beetles (11,000 specimens), which were in different stages from egg to imago, did not change. The number of mutations increased, but the increase was statistically unreliable; however, among the resulting mutations there occurred some not previously described [69]. Exposure to space of poliomyelitis virus and T-even phages with minimum shielding (38  $\mu$ m Al) resulted in slight inactivation of the virus and phages. No other changes were observed [53-54]. The exposure was at altitudes of 80-155 km.

The results of sounding of the lower radiation belt by a rocket carrying neurospora in Project NERV were inconclusive in many respects [70-71]. The rocket rose approximately vertically to an altitude of 1900 km and remained within the radiation belt for 28 min. The experimental material showed 200 times more mutations than the control. Physiological deviations also were noted; these included decrease in the concentration of substances necessary to energy metabolism. The low survival (3.2%) of the spores probably indicates that the maintenance conditions of the neurospora during flight were unfavorable. Information on the radiation conditions is not included in the reports.

In 1958-1960 ballistic rockets carrying mice and monkeys were launched in the United States. In some cases the launchings were successful. Besides the mammals, on some flights the rockets carried the eggs and sperm cells of sea urchins, yeast cells, lower fungi, *E. coli*, onion bulbs, seed corn, mustard seed, and *Drosophila* [72-77]. No significant genetic changes were detected in the experimental objects. There was a decrease in the viability of fertilized sea urchin eggs, but this result was later identified as artefact [76]. No detailed exposition of the results of these experiments has been published.

### 3. Experiments on Artificial Earth Satellites

The number of genetic investigations in space began to increase rapidly especially after the development of recoverable artificial earth satellites. The very first such object, the second Soviet orbital spacecraft, might well be compared with Noah's ark. It carried 26 species of animals, plants, and micro-/145 organisms, many species being represented by several lines or strains. The great volume of experimental material makes it desirable to list the experimental objects by taxonomic classification.

A. Microorganisms. The most extensive investigations were those on induced phage production in lysogenic strains of *E. coli* K-12 ( $\lambda$ ) due to space-flight factors. This object was exposed on more than ten artificial earth satellite flights **varying** in duration from 1.5 hours to 3 weeks [78-87]. Usually when the flight lasted more than a day there was a small but statistically quite reliable increase in phage production in the experimental material. However, correlation between the magnitude of the effect and the duration of the flight was far from consistent. In all experiments in which the effect was noted, it was greater than would have been predicted for exposure to cosmic radiation, based on the doses measured in flight and the character of the dose-effect relationship. The authors feel that the effect is not an artefact but was caused by the combined effect of cosmic radiation and other flight factors.

In addition to the study of phage production in *E. coli* K-12 ( $\lambda$ ), a research team headed by N. N. Zhukov-Verezhnikov conducted experiments with *Aerobacter aerogenes* 1321, *E. coli*, *Staphylococcus aureus* 0-15, and *Clostridium butyricum*. Viability of bacteria in the experimental and control samples was approximately identical [78-80, 86]. The last object (*Cl. butyricum*) was flown both in spore suspensions and in bioelements. No changes in viability were observed when the bacteria were sown on a nutrient medium during flight. The 1321 and T-2 phages were flown on several artificial earth satellite experiments. Space flights had no effect on the number of viable phage particles [78-80]. Spaceflight factors also had no effect on several strains of tobacco mosaic virus and grippe virus [88-89].

The incidence of biochemical mutations in *E. coli* did not change following spaceflight [78,86]. However, dissociative forms with higher mutability and different biochemical **properties** were observed in *E. coli* [90]. An attempt to induce dissociative transitions in *Bacillus brevis*, whose spores were carried on the "Voskhod" flight, gave negative results [91].

Use of the radioprotector  $\beta$ -mercaptopypylamine blocked induced phage production in *E. coli* and also appreciably reduced spontaneous phage production. This substance had no effect on the viability of bacteria in spaceflight [83,85].

The death of a considerable part (up to 50%) of yeast haploid cells, sensitized by low concentrations of oleic acid, was noted on the flight of the orbital spacecraft "Vostok-2". Flight had no effect on the diploid cells or unsensitized haploid cells [92-93]. This test was repeated on the flight of "Voskhod-1". For diploid cells the results were fully confirmed, but the results for haploid cells were contradictory: in one experimental series the effect was present

and in the other it was absent [94]. The results of experiments with yeasts were remarkable in two respects. Firstly, they show that sensitization of biological objects may be a useful method of increasing the sensitivity of the tests, and secondly, it complicates the difficulties in analysis of any effect which occurs.

On a number of spaceflights (second, fourth and fifth orbital spacecraft) a study was made of spore viability and some growth characteristics of several strains of *Actinomyces* with various radio sensitivity [95-96]. The data on spore viability were contradictory. Compared to the controls, viability of the experimental spores decreased in some strains but increased in others. At the same time, there was pronounced stimulation of mycelium growth following germination of the flown spores. Similar phenomena were later noted more than once in various plant objects. /146

In the experiment on the second orbital spacecraft flight factors had no significant effect on the general viability of *Chlorella* cultures [97]. The lethality of individual *Chlorella* cells was not taken into account in this experiment. A more detailed genetic study of *Chlorella* was made in other studies [98, 99]. These experiments took into account cell viability, the reproduction rate, and the incidence of chlorophyll and morphological mutations in several strains of three species of *Chlorella*. As a rule, sporulation lagged somewhat following flight. After repeated flights mutation frequency showed a slight increase. Approximately the same results were obtained with *Chlorella* in an experiment on "Discoverer 17" [100-101]. Spores of *Clostridium sporogenes* were carried in addition to *Chlorella* on "Discoverer 17". The experimental spores withstood incubation in caramelized glucose more poorly. Their viability was only about 30%. A decrease of the viability of nonincubated spores was also noted, but this was probably an artefact caused by spore adhesion [102]. An experiment with spores of *Clostridium sporogenes* and neurospora also was carried out during the flight of "Discoverer 18" [103]. The effect of incubation in caramelized glucose was considerably less pronounced (viability 88%). It will be recalled that the flight of "Discoverer 17" took place during a solar flare and the biological objects on board received a dose of 20-33 rad, largely accounted for by protons.

B. Higher plants. A large number of studies were made during artificial earth satellite flights with dry seeds of various higher plants. In most of these experiments the seeds were caused to germinate after the return of the artificial earth satellite and determination was made of the frequency of various types of chromosome rearrangements in primary roots and at growth points. In some cases the mitotic index was determined.

B. Sidorov and N. Sokolov [104,105], working with *Allium fistulosum* L. and *Nigella*, which are very different in radiosensitivity, found no differences between experimental seeds carried aboard the second and fifth orbital spacecraft and "Vostok-1" and the controls. The use in the experiments of wetted seeds and seed sprouts with a radiosensitivity 20-30 times greater, was also of no help in detecting a genetic effect. Incidentally, an effect of stimulation of the germination process and of germination energy, particularly clear in the radioresistant *Nigella*, was observed when the seeds were caused to germinate.

In studies using diploid and tetraploid buckwheat there were likewise no differences between experimental and control specimens [106]. This object was carried aboard "Vostok 1", "Vostok 2", "Vostok 3", and "Vostok 4". The incidence of chromosome aberrations in violet stock (gillyflower) showed no increase following a 1 1/2-hour space flight [107].

Contradictory data were obtained on chromosome rearrangements in wheat and several varieties of peas, which were used in a considerable number of experiments [95-96,108]. In three cases, statistically reliable differences between experimental and control specimens were observed in wheat, but in six cases there were no differences or they were statistically unreliable. In the case of peas, statistically reliable increases of the incidence of chromosome aberrations were observed in four out of ten cases. There was no correlation between the /147 duration of flight and the presence of a genetic effect in the case of either biological object. At the same time, chromosome-type aberrations predominated in the experimental material, while most of the aberrations in the controls were of the chromatid type, as should be true for the spontaneous mutation process. Mitotic index data are also contradictory: in some cases the mitotic index increased and in other cases it decreased. Some experiments showed an intensification of seed germination energy although the mitotic index remained constant. Thus, spaceflight possibly stimulates plant growth not only by intensifying the rate of cell division, but also by increasing cell volume.

In addition to seeds of wheat and peas, similar postflight studies were made on the seeds of carrot, bean, pine, cucumber, tomato, lettuce, and mustard, carried on several flights [99,109-110]. Generally speaking, a post-flight increase in the incidence of chromosome rearrangements was noted in almost all species studied. However, the effect was small in absolute terms and therefore was seldom statistically reliable. About 100 cells were usually analyzed in the experimental and control series. The maximum effect was observed in carrot and tomato seeds. In the carrot seeds the percentage of induced aberrations was more than 5%; in the tomato seeds it was about 4%. It is noteworthy that there is no correlation either between magnitude of effect and flight duration or between magnitude of the effect and radiosensitivity of the plant species. In these species, as in wheat and peas, the genetic effect was attributable to crease in chromosome rearrangements. The incidence of chromatid rearrangements in the experiment and control specimens was almost identical.

Studies of the effect of spaceflight factors on the germination, sprouting, and intensity of development of the seeds of different plants are of considerable interest [99,109,111]. Appropriate experiments were made during the flights of "Vostok 3", "Vostok 4", "Vostok 5" and "Vostok 6". In many species the experimental specimens manifested stimulation of the indices, but in several cases inhibition was observed. In these experiments a nongenetic effect was discovered, but the experimental results indicate that spaceflight factors indirectly affect the processes of cell division, which are inseparably connected with chromosome behavior. Histochemical studies revealed no changes in the concentration of proteins, fats, starch, and individual amino acids. In cases where growth stimulation occurred there was an increase in ascorbic acid concentration and reduction enzyme activity [99,109,111].

In several experiments spindle-tree seeds were exposed to preliminary  $\gamma$ -ray irradiation [112]. An additive effect was observed, that is, the preliminary irradiation and space flight factors independently affected the incidence of chromosome rearrangements. The flight of "Vostok 5" also showed reliable differences between experimental and control specimens of intact spindle-tree seeds.

H. Curtis and H. Smith determined the incidence of somatic chlorophyll mutations in corn flown on "Discoverer 32" (27 hours) and on a secret satellite (50 hours) [113]. The experiment was backed up by a photoemulsion control. There was a small but statistically reliable increase in mutation frequency, which agreed with that predicted from computed data. The high resolution of this method is noteworthy, since mutation frequency increased by only 0.01-0.001 per leaf and this increase was successfully registered.

Much material is now available on the effect of spaceflights on chromosome rearrangements and also on the mitotic process and growth processes in micro- /148 spores of *Tradescantia paludosa* (spiderwort). Appropriate experiments were conducted on the flights of the satellites "Vostok 3", "Vostok 4", "Vostok 5", "Vostok 6" and "Voskhod". Cosmonauts P. Popovich, V. Bykovskiy and B. Yegorov took active part in these experiments [99,114-121]. In all the experiments conducted with methodological correctness, cytological analysis showed increased incidence of chromosome rearrangements, the appearance of anomalous mitoses, and impairment of growth processes. The experimental conditions, times of fixing the material, and the results obtained were related in such a way as to cause the investigators to conclude that chromosome rearrangements are principally due to factors operating during the launch and re-entry of the artificial earth satellite. The appearance of anomalous mitoses and the impairment of growth processes are most probably caused by weightlessness or the combined effect of weightlessness and cosmic radiation. However, the investigators do not consider either hypothesis to be conclusively proven.

The experimental material displayed a new type of chromosome aberration not occurring after exposure to ionizing radiations. These aberrations consist of spherical fragments resembling nucleoli; they are probably formed by the adhesion of several fragments into a single unit. Spherical fragments are found in all stages of the cell cycle.

C. Animals. Experiments *in vivo* and *in vitro*. Studies of *Drosophila* were conducted extensively on artificial earth satellite flights. The incidence of recessive lethal mutations in the sex chromosome of males was determined during the flights of the second, fourth, and fifth spaceships: "Vostok 1", "Vostok 2", "Vostok 3", "Vostok 4", and "Voskhod" [122-127]. The experiments were usually conducted on two lines of *Drosophila melanogaster*, with high and low mutability. When possible, i.e. when flight did not last too long, embryo cells were analyzed separately at the sperm maturation and spermatid stages. A statistically reliable increase in the incidence of recessive lethals was noted in three experiments: on the second and fourth orbital spacecraft and on "Vostok 1". In four other experiments no effect was noted. No correlations were established between flight duration and the magnitude or even the presence of genetic effects. It is probably correct to say of this test that there is a negative correlation between genetic effect and flight duration.

Cytological analysis of the chromosomes of the salivary glands was made when mutation incidence increased and showed them to be point mutations. The exposure of *Drosophila* at different stages (eggs, larvae, imago) did not influence the nature of the effect [126,127]. The use of the radioprotector 5-methoxytryptamine had no effect on the incidence of mutations [127].

With respect to primary nonseparation of sex chromosomes in female *Drosophila*, reliable differences between experimental and control specimens were obtained in two cases. Nearly reliable differences were obtained in another experiment. In still another experiment no differences were noted. Again, for this test the correlation between the observed effect and duration of flight is at least externally negative. The maximum effect was observed on the flight of "Vostok 1" (duration 1.5 hr) and the minimum was observed for the flight of "Vostok 3" (duration 96 hr). This relationship renders improbable even a cautious assumption that nonseparation of chromosomes is induced by weightlessness [123,128]. In making these experiments the authors noted two interesting peculiarities: the effect did not depend on the stage of gametogenesis, and the male-to-female sex ratio obtained was that characteristic of spontaneous nonseparation of chromosomes.

Eight experiments were carried out to determine the incidence of dominant /149 lethal mutations in the gametes of male *Drosophila* following spaceflight [123, 129,130]. In three of them two lines of *Drosophila* were used, and in nine cases mutability was analyzed separately at the sperm maturation and spermatid-spermatocyte stages. The most significant results of these experiments are as follows: the percentage of induced dominant lethals was positive and small in all the experiments; there was no correlation between the magnitude of the effect and flight duration; the frequency of lethals in the line with high mutability was somewhat less than in the line with low mutability; in the spermatids-spermatocytes the effect was more pronounced than in mature sperm. Based on additional laboratory experiments [123,129,131] it was concluded that the observed effect was due to decreased fertility of the males caused mainly by nonspecific spaceflight factors. There is no adequate basis for assuming that induction of an appreciable incidence of mutations of the dominant lethal type occurred during spaceflight.

A high egg mortality following spaceflight was observed in experiments using female *Drosophila* [130]. However, this effect was virtually nonexistent in cases when the females were cross-bred prior to launch. Thus, in this case also the result was not caused by induced dominant lethals, but rather by impaired sperm utilization, which investigators feel was due to temperature shocks.

A fourth type of genetic change observed in *Drosophila* following spaceflights was crossing over in the germ cells of males [123,132]. A statistically reliable effect was observed in one experiment out of three. Absence of any effect in two flights may be attributed to the fact that in analyzing the gametes, the stage of late spermatogonia, in which crossing over occurs in males most frequently, was overlooked. Laboratory investigations showed that crossing over may be induced in male *Drosophila* by low-frequency vibration [133].

On the flights of "Vostok 3" and "Vostok 4" the cosmonauts crossbred virgin

female *Drosophila* with males after the satellites were put into orbit [126]. These experiments showed that copulation, egg laying, embryo and larval development of *Drosophila* may occur normally under conditions of weightlessness. The appearance of morphoses was noted, but their character was nonspecific and the difference in incidence from the controls was not statistically reliable. In this connection it should be mentioned that no anomalies in embryo development were detected following exposure of horse ascarid eggs on artificial earth satellites [134].

Cytogenetic experiments with mice were conducted on the flights of three artificial earth satellites, the second, fourth and fifth orbital spacecraft. After landing the incidence of chromosome rearrangements in bone marrow and spleen was determined. In the first two experiments a statistically reliable effect was found, while in the third there was no effect [135-138]. The effect was distinguished by its great persistence and by the almost complete fragmentation of the chromosomes. The nature of the damage indicated that it was probably caused by adhesion and subsequent incorrect separation of the chromosomes, rather than by chromosome breakage. A similar effect occurs when animals are exposed to vibration [138-142].

In experiments on cultures of normal and pathological human cells (He-La clones, fibroblasts, and human amniotic cells), which were carried out during the flights of the second, fourth and, fifth orbital spacecraft and on "Vostok 1", "Vostok 2", "Vostok 4" and "Vostok 6", the cells in the monolayer cultures on glass were intact and cell capacity to grow and reproduce was unimpaired on re-150 turn to normal conditions. In a number of experiments some immunobiological changes occurred. Chromosome aberrations were not counted in these experiments [78-79,85-86]. In several experiments on Soviet artificial earth satellites, the viability and healing rate of human and rabbit skin autotransplants following exposure to space were studied. The data obtained indicate that skin grafts retain viability [78-79,88-89].

Experiments conducted on the artificial earth satellites "Discoverer 17" and "Discoverer 18" showed an activity increase in antigen-antibody reactions similar to that caused by chemical mutagens or ionizing radiation [143-144]. However, the investigators were able to evoke a similar effect by exposure to vibrations. Cells of various human tissues -- the amnion, conjunctiva, synovial sheath, bone marrow, leukemic monocytes, embryo lung -- were exposed on these same artificial earth satellites in monolayer cultures. Poor maintenance conditions caused degeneration and death of a considerable part of the cells in both the experimental and control material. The experimental material displayed multipolar mitoses, haploid, polyploid, and aneuploid nuclei, and fragmented and dicentric chromosomes, but the author gives no numerical data on these phenomena [145-146].

Analysis of cultures of brain cells from 10-day old chicken embryos, exposed to space on "Discoverer 18", showed that cell viability in the material which was only transported to the launch site was lower than in the material which had been flown [147]. This was apparently due to inadequate maintenance conditions in the prelaunch period.

A refined cytogenetic experiment was conducted on a leukocyte culture from peripheral blood exposed *in vivo* and *in vitro* during the flight of "Gemini 3" [148-150]. Cultures of leukocytes flown on the satellite and left behind at the launching pad were exposed to  $\beta$ -irradiation in doses up to 180 rad. In the experimental cultures the incidence of two-hit chromosome aberrations (rings and dicentrics) was twice as great as in the controls. The incidence of one-hit aberrations (chromosome and isochromatid deletions) was approximately the same. In the experiment *in vivo*, i.e., with pre- and post-flight examination of the astronauts, the incidence of all types of aberrations was identical. To be sure, the experiment *in vivo* was made without additional irradiation. The experimental results show that weightlessness does not initiate chromosome aberrations, but does affect the course of the pre-existing aberrations, tending to increase the number of reunions due to chromosome restitution.

The results of genetic investigations during the flights of artificial earth satellites may be summarized as follows. For a number of objects -- *Chlorella*, tissue cultures (nonirradiated), phages and viruses, some bacteria, dry seeds, and some species of plants -- not a single flight produced significant differences between experimental and control specimens. In other experiments, for example, those on wheat, peas, *Drosophila* and mice, the results are contradictory. Following some flights a perfectly reliable genetic effect was observed, while following others it was absent. Furthermore, no relationship was observable between the magnitude of the effect on the one hand, and the duration of the flight on the other. In the third group of objects and tests, particularly those involving phage production in *E. coli*, and also chromosome rearrangements and mitotic disturbances in *Tradescantia*, the results were more or less uniform, permitting the investigators to make a preliminary causal analysis of the data obtained. Finally, it is possible to distinguish a fourth group of objects which showed a clear genetic effect in response to specific research methods [113,148-150]. /151 These experiments, however, were only made on single flights and results require further confirmation. Such a diversity of results stems from a number of circumstances associated with the effect of mutagenic factors in the experiments and the conditions under which they were carried out. In space flight the biological objects are exposed not only to cosmic radiation, but also to a whole series of other factors. Since existing theories of radiation mutagenesis are universal, although of course still incomplete and in part contradictory, the result of the effect of ionizing radiation on biological objects may be roughly predicted.

This sort of prediction cannot be made for other physical factors, primarily because the mechanism by which their energy is transmitted to the nuclear structures of cells is unknown, if such a mechanism in fact exists. It is this fact, together with the low (usually tenths of a rad) doses of cosmic radiation encountered during the flights and the known sensitivity of the methods, which has led the investigators to attribute even the consistent results to combined effects (phage production), or to the action of dynamic flight factors (chromosome rearrangements in *Tradescantia*), or to the action of weightlessness (impaired mitoses in *Tradescantia*).

In those cases when no genetic effects are observed for a whole series of flights, it is evident that the sensitivity of the research methods is too low

to register the genetic changes induced by combined effects. Of course, the mounting of such studies must be excused since the objects in question were flown on the first artificial earth satellites when virtually nothing was known concerning the biological effect of flight factors.

It should be noted that the absence of effect due to the poor sensitivity of the experimental method may sometimes lead to unjustifiably optimistic conclusions.

The contradictory results obtained for a number of objects during spaceflight may in our opinion be attributed to three interrelated factors: first, the small difference between the magnitude of effect induced by spaceflight factors and the sensitivity of the experimental methods; second, differences in the maintenance conditions for the experimental and control materials, which are not only irrelevant to spaceflight itself, but which varied from experiment to experiment; and third, lack of genotype uniformity in the material used either for any one experiment or (especially) for different experiments.

That the first factor is a real one is shown by the fact that although theoretically no dose threshold exists for the appearance of mutations, not a single investigator has yet studied *Drosophila*, for example, with doses smaller than 1 rad. The reality of the second factor is indicated both by the great differences between control specimens remaining in the laboratory and those transported to the launch site and back, and also by the differences between control specimens transported to the launch site and back in different experiments. The reality of the third factor is indicated by the high incidence of mutations in the control material remaining in the laboratory, and the considerable fluctuations in this incidence observed when laboratory control series of a number of objects were bred.

Glembotskiy and associates [126], analyzing the results of seven spaceflights, noted that the reaction of inherited structures in different objects to the conditions of a particular spaceflight tended to be parallel, while the reactions to the conditions of different flights varied greatly, this variation having no correlation with flight duration. Correctly pointing out that this /152 phenomenon may be due to variation in some flight factor, the authors suggest very cautiously that the source of this variation is related to cosmic radiation, particularly to the little studied heavy component. This explanation has two shortcomings. The less important of these is the difficulty of demonstrating the statistical reliability of the observed parallelism, which is to say its existence. The more important objection is that cosmic radiation varies in rigorous correlation with flight duration, so that the genetic effect should show the same correlation, which in actuality it does not. For these reasons, our own explanation of the contradictory nature of the results obtained for several test objects appears to us to be the more sound.

Of course, other explanations of the obtained results are also possible. For example, it might be suggested that the elimination of mutant germ cells is intensified in *Drosophila* under conditions of weightlessness, or that prolonged exposure to weightlessness changes the dynamics of germ cells to bring the least radiosensitive stages under analysis. Explanations of this kind have a common

shortcoming -- they postulate the existence of phenomena whose reality is doubtful.

Thus, the successful conduct of genetic experiments during spaceflights inside the earth's radiation belts requires that biological objects be found and research methods developed providing a high degree of sensitivity, and that variability due to nonuniform maintenance of the experimental and control specimens be eliminated, and finally, that genetically uniform material be used in carrying out the experiments.

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